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Developmental regulation of murine mammary-gland 90 kDa heat-shock proteins

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We have examined the regulation of murine mammary-gland 90 kDa heat-shock protein (hsp-90) as a function of normal development and differentiation. We find that both hsp-90 and amounts of its mRNA are modulated during development and differentiation, with the highest concentrations of mRNA and protein being present in tissues from pregnant and lactating animals respectively. Metabolic labelling experiments with [^{35}S]methionine reveal that the rate of synthesis of hsp-90 also varies among tissues from various developmental states and correlates with the relative hsp-90 mRNA content. These data also suggest that the highest concentration of hsp-90 found in lactating mammary tissues may be due to a greater stability of this protein in this developmental state. The possible significance of the developmental modulation of mammary hsp-90 to mammary steroid-receptor properties is discussed.

INTRODUCTION

In previous studies in our laboratory [1], we have documented that the oestrogenic sensitivity of normal mouse mammary tissues is modulated during development and differentiation, such that during lactation mammary glands are unresponsive to oestradiol despite the presence of oestrogen receptors. We have also documented that, in contrast with oestrogen receptors isolated from oestrogen-sensitive mammary tissues, the ability of oestrogen receptors in lactating mammary glands to bind to DNA or chromatin *in vitro* is impaired [2,3]. In addition, our studies on oestrogen receptors binding to DNA and chromatin also revealed that the impeded interaction of oestrogen receptors in lactating mammary tissues may be due to some endogenous inhibitor(s).

Recent studies from several laboratories have demonstrated that steroid receptors *in vitro* may complex with cellular 90 kDa heat-shock proteins (hsp-90), and that this association can render the receptor in a physical state unable to bind to DNA [4–8]. It is well documented that mammary development and differentiation is under multi-hormonal control, including the ovarian hormones oestrogen and progesterone [9]. In avian oviduct, ovarian hormones have been shown to regulate at the transcriptional level the synthesis of a 108 kDa protein related to the family of heat-shock proteins ('hsp-108') [10]. In recent studies in our laboratory we have found that oestrogens can stimulate the synthesis of hsp-90 in target tissues for oestrogen in rodents [11]. Accordingly, it seemed reasonable to speculate that the cellular factor(s) in lactating mammary tissue impeding the interaction of oestrogen receptors with DNA and chromatin may correspond to the hsp-90, and, if this were so, perhaps the concentration of this protein during lactation was higher than that present in non-lactating tissues. Such a possibility was also strengthened by observations from other laboratories, which had revealed that in murine tissues the synthesis of hsp-90 was modulated as a function of differentiation [12,13]. Although it is well known that hsp-90 is a ubiquitous and an abundant cellular protein, as yet its presence in normal murine mammary tissues has not been demonstrated. Therefore in the present studies we have examined the developmental regulation of hsp-90 in normal murine mammary glands.

EXPERIMENTAL

Animals

Female Balb/c nulliparous mice, mice in mid-gestation and lactating mice between days 7 and 10 *post partum* and days 28–30 *post partum* (involuting) were used in these studies.

Tissue preparation

All procedures were carried out at 0–4 °C. The cytoplasmic extracts were prepared either in PG buffer [5 mM-sodium phosphate, 10 mM-monothioglycerol and 10% (v/v) glycerol, pH 7.4 at 23 °C] or in TED buffer (10 mM-Tris, 1.5 mM-EDTA, 1 mM-dithiothreitol, pH 7.4) with protease inhibitors (aprotinin 77 μg/ml, leupeptin 0.1 mM, bacitracin 100 μg/ml, pepstatin 1 μg/ml) in accordance with procedures previously described [2]. Immediately after preparation, the cytoplasmic extracts were used either as such or after being frozen in liquid N_2 and stored at −70 °C.

Preparation of [^{35}S]methionine-labelled tissue extracts

Mammary tissues from various developmental states were incubated at 37 °C in a methionine-free Eagle's minimum essential medium containing 25 μCi of [^{35}S]methionine (1100 Ci/mmol)/ml for 90 min under

Abbreviation used: hsp-90, 90 kDa heat-shock protein.

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an atmosphere of air/CO_2 (19:1). At the end of incubation, tissues were rinsed three times in Eagle's medium before homogenization and preparation of extracts.

Description of antibodies and cDNA probes

Hsp-90 A_1. This is a rabbit antiserum prepared against a synthetic peptide corresponding to a defined region (amino acids 260–280) derived from the avian hsp-90 cDNA sequence [14] and known to cross-react with hsp-90 from a number of species, including mammals [15].

AC88. This is a mouse monoclonal antibody prepared against the 88 kDa protein purified from the aquatic fungus *Achlya ambisexualis*, exhibiting cross-reactivity with avian and mammalian hsp-90 [16].

L2. This is a rabbit antiserum raised against the immunoaffinity purified hsp-90 from avian oviduct cytosol [8], cross-reacting with both avian and mammalian hsp-90 [17].

B2. This is a rabbit antiserum raised against partially purified hsp-90 from calf uterine cytosol, cross-reacting with both avian and mammalian hsp-90 [17].

cDNA probes. A murine hsp-90 partial cDNA clone (H-19) corresponding to the 'heavy' thermoinducible form of hsp-90 [18] was used in these studies. Murine β-actin cDNA clone was a gift from Dr. Margaret Buckingham (Institut Pasteur, Paris, France). Whey acidic protein (WAP) cDNA clone was obtained from Dr. L. Hennighausen (National Institutes of Health, Bethesda, MD, U.S.A.).

Gel electrophoresis and immunoblotting

The cytoplasmic extract was made up in SDS sample buffer to a final concentration of 135 mM-Tris/HCl (pH 6.8), 2% (w/v) SDS, 10% glycerol, 5% (w/v) 2-mercaptoethanol and 0.05% Bromophenol Blue, and the sample was heated in a boiling-water bath for 5 min. A portion of the sample was separated by electrophoresis in a discontinuous SDS/polyacrylamide gel containing 7.5% acrylamide/0.075% methylenebisacrylamide in the resolving gel and 3% acrylamide/0.08% methylenebisacrylamide in the stacking gel. In some initial experiments the resolving gel contained 10% acrylamide instead of 7.5%. The buffer system used was Laemmli's [19]. After electrophoresis, the gel was electroblotted on to nitrocellulose by using Tris/glycine buffer, as described by Towbin *et al.* [20]. The hsp-90 in the blot was probed with various antisera and antibodies and the commercially available biotinylated anti-mouse or anti-rabbit IgG and avidin–biotinylated peroxidase. The blot was processed in accordance with the manufacturer's instructions (Vectastain kit, from Vector Laboratories, Burlingame, CA, U.S.A.). In some experiments, instead of the Vectastain kit, ^{125}I-Protein A (0.1 μCi/ml) was used to identify hsp-90 by autoradiography. When ^{125}I-protein A was used, the band corresponding to hsp-90 was cut out and counted for radioactivity in a γ-counter to quantify the amount of protein.

RNA preparation, Northern and slot-blot analysis

Total RNA from mammary glands from various developmental states was prepared by modification of standard procedures [21]. In short, tissues were pulverized in liquid N_2 and homogenized in a 1:1 (v/v) mixture of phenol and buffer containing 20 mM-Tris, pH 7.5, 1 mM-EDTA and 2% SDS. The aqueous phase was extracted once with phenol and twice with phenol/chloroform (1:1, v/v), and then treated with DNAase I and a subsequent extraction with phenol/chloroform, before precipitation of RNA with 50% (v/v) ethanol. The integrity of the RNA was checked by electrophoresis on 1.4%-agarose gels [22]. For Northern analysis, 20 μg of RNA was fractionated by electrophoresis in denaturing 1%-agarose gels containing 2.2 M-formaldehyde, and transferred to nitrocellulose in accordance with standard procedures [21]. For slot-blot analysis, known amounts of RNA were applied on to nitrocellulose filters by using a Minifold II apparatus (Schleicher and Schuell). The filters were baked at 80 °C for 2 h under vacuum and prehybridized at 42 °C for 2 h exactly as described in ref. [22]. The RNA on the filters was then hybridized at 42 °C overnight to the various cDNA clones which

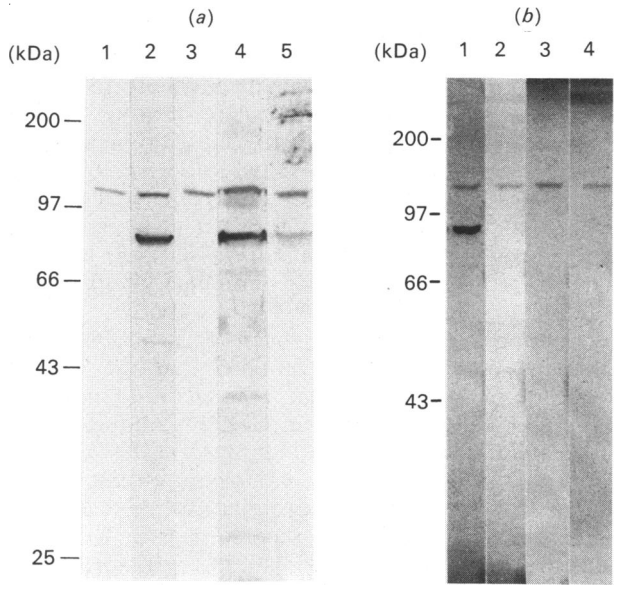

Fig. 1. Detection of hsp-90 in lactating-mammary-gland cytosol with various anti-hsp-90 antibodies

Portions of lactating-mammary-gland cytosol were subjected to electrophoresis on SDS/10% polyacrylamide gels, immunoblotting and colour development by using a Vectastain kit, as described in the text. (*a*) Lanes 2, 4 and 5 were incubated initially with AC88 antibody and B2 and L2 antisera respectively, followed by either biotinylated anti-mouse IgG (lane 2) or biotinylated anti-rabbit IgG (lanes 4 and 5). Strips corresponding to lanes 1 and 3 were treated only with biotinylated anti-mouse IgG and anti-rabbit IgG respectively. (*b*) Lane 1 was probed with antiserum hsp-90 A_1 (diluted 1:300); lanes 2 and 3 were probed with the same amount of antiserum, but in the presence of the synthetic peptide (which was used as immunogen) at 1 and 10 μM respectively. Lane 4 was probed with non-immune rabbit serum. Subsequently all lanes were developed with biotinylated anti-IgG and avidin–biotinylated peroxidase as described in the text.

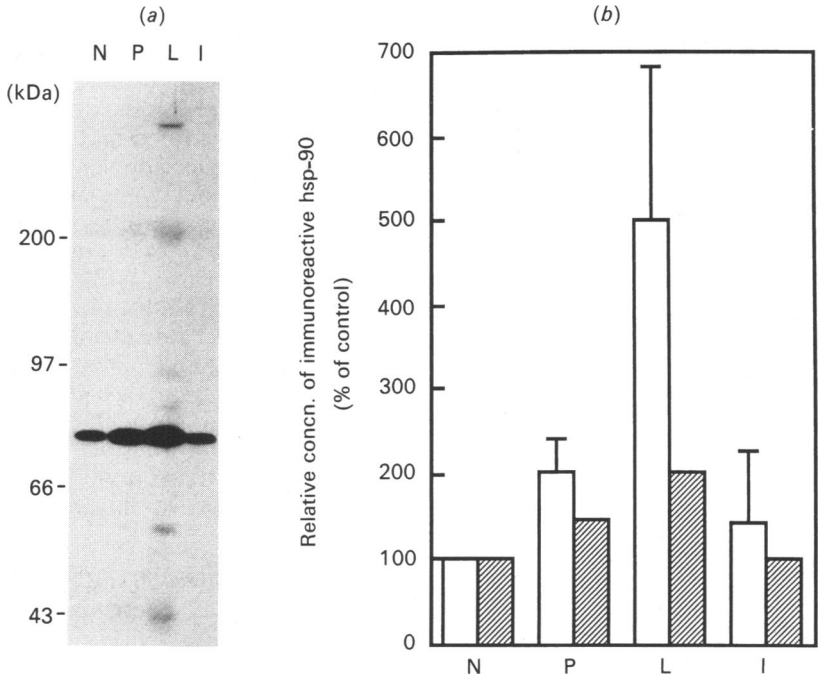

Fig. 2. Relative concentrations of hsp-90 in mammary tissues from various developmental states

Equal samples of mammary cytosol from the various developmental states (N, nulliparous; P, pregnant; L, lactating; I, involuting) were processed for measurement of hsp-90 by using antiserum hsp-90 A_1 and ^{125}I-Protein A as described in the Experimental section. (*a*) Autoradiograph of a typical immunoblot. (*b*) Amounts of hsp-90 present in equal volumes of the extracts, as measured (□), and normalized to total protein concentration (▨). The data represent the averages of four separate experiments. The 100% control value represents the amount of hsp-90 in mammary cytosol of nulliparous mice.

had been nick-translated to a specific radioactivity of $(2–4) \times 10^8$ c.p.m./μg of DNA by using [^{32}P]GTP, [^{32}P]CTP and commercially available kits (BRL). The blots were then washed under high stringency (60 °C, in 15 mM-NaCl/1.5 mM-sodium nitrate), dried, and autoradiographed [22]. The relative intensity of the autoradiographic bands was determined by scanning densitometry (LKB 2200 densitometer) to quantify the relative mRNA concentration (hybridized to the ^{32}P-labelled probe) in each sample.

DNA, RNA and protein assays

Total tissue extracts were analysed for concentration of DNA as described by Ceriotti [23], for RNA by the orcinol colour reaction [24], and for protein by the method of Bradford [25].

RESULTS

Demonstration of hsp-90 in mammary tissue extracts

Although it is generally believed that heat-shock proteins are ubiquitous in Nature, as yet the presence of hsp-90 in normal murine mammary tissues has not been demonstrated. Therefore in our initial studies we analysed the lactating-mammary-gland cytosol for the presence of hsp-90 by immunoblot assays. As shown in Fig. 1(*a*), a band exhibiting cross-reactivity with a variety of anti-hsp-90 antibodies and corresponding to an approximate molecular mass of 90 kDa was apparent; the calculated molecular mass of this band was 88 kDa. In these experiments (Fig. 1*a*) an additional major band corresponding to ~ 130 kDa was also visible, which was not antibody-specific (see lanes 1 and 3). Similar data were also obtained with experiments in which a rabbit antiserum (hsp-90 A_1) to a synthetic peptide corresponding to a defined region of avian hsp-90 had been used for immunoblotting (Fig. 1*b*, lane 1). This antiserum against the synthetic peptide (hsp-90 A_1) recognizes the 90 kDa heat-shock protein isolated from heat-shocked HeLa-cell lysates [26]. The immunoreactive band corresponding to 90 kDa was not visible when either non-immune serum or antibody incubated with an excess of antigen (the synthetic peptide) was used (see lanes 2–4, Fig. 1*b*). The band corresponding to ~ 130 kDa was present in all lanes, thereby indicating once again its non-specificity. Owing to its cross-reactivity with a variety of anti-hsp-90 antibodies, and also because in general heat-shock proteins, including hsp-90, can be synthesized constitutively [27], the 90 kDa band shown in Fig. 1 most likely represents mammary hsp-90.

Modulation of hsp-90 amount during mammary development and differentiation

An analysis of cytoplasmic extracts of mammary tissues isolated from various developmental states (Fig. 2*a*) revealed that the amount of hsp-90 was higher in tissues isolated from pregnant and lactating mice (lanes P and L respectively) compared with that present in tissues derived from either nulliparous mice or tissues undergoing lactational involution (lanes N and I respectively). Similar profiles were obtained with two other antibodies recognizing hsp-90, namely AC88 and B2 (results not shown). An analysis of the steady-state amount of hsp-90 relative to total cytosolic protein shown in Fig. 2(*b*) reveal that the amounts of hsp-90 in

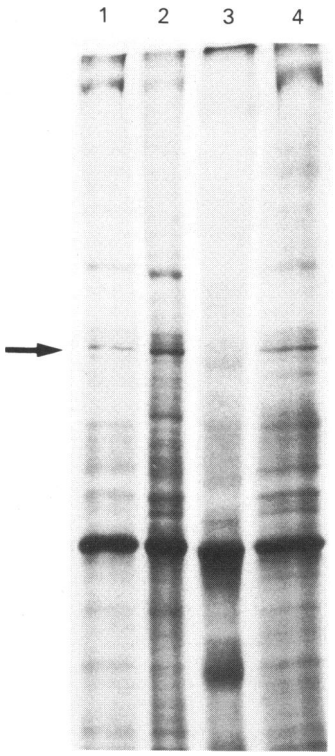

Fig. 3. Comparison of the pattern of hsp-90 synthesis in mammary tissues from various developmental states

Tissues were incubated in a medium containing [^{35}S]-methionine as described in the Experimental section, and processed for analysis of hsp-90. Duplicate samples of tissue extracts from nulliparous (lane 1), pregnant (lane 2), lactating (lane 3) and involuting (lane 4) animals containing equal amounts (~ 200000 c.p.m.) of trichloroacetic acid-precipitable radioactivity were subjected to SDS/10%-polyacrylamide-gel electrophoresis. Subsequently one blot was processed for immunoblotting with the Vectastain kit to locate hsp-90 (indicated by the arrow), and the parallel blot was subjected to autoradiography. Fig. 3 shows the autoradiograph of the blot.

mammary tissues of nulliparous mice and those undergoing lactational involution are not significantly different from each other; however, during pregnancy there is an approx. 2-fold increase in mammary hsp-90, and a 5-fold increase during lactation. The increase in total tissue concentration of hsp-90 during pregnancy and lactation is also apparent when the data are normalized to total protein concentration (Fig. 2*b*). Similar differences, but of a lesser magnitude, were also apparent if the data were normalized to the DNA content of the tissue; based on 100% control value for the concentration of hsp-90 (normalized to DNA) for tissues from nulliparous mice, the amounts of hsp-90 in tissues from pregnant, lactating and involuting mice were 129%, 226% and 103% respectively.

Next, to determine if the different amounts of hsp-90 present in the various cytosols corresponded to their relative rates of synthesis by the tissues from various different developmental states, metabolic labelling experiments with [^{35}S]methionine were performed. In initial experiments it was verified that the incorporation of [^{35}S]methionine into total acid-precipitable radioactivity was linear with time up to 3 h of incubation (results not shown). Direct electrophoresis of labelled tissue extracts containing the same amount of acid-precipitable radioactivity followed by immunoblotting to locate hsp-90 by using a Vectastain kit and then followed by autoradiography revealed that the mammary tissues isolated from different developmental states had different rates of incorporation of [^{35}S]methionine into hsp-90 (Figs. 3 and 4); the relative intensity of the autoradiographic bands corresponding to hsp-90 revealed that, as compared with tissues from control (100%) nulliparous mice (lane 1, Fig. 3, and panel *a*, Fig. 4), the tissue from pregnant mice had the highest rate (approx. 35% more than control; lane 2, Fig. 3, and panel *c*, Fig. 4), whereas the tissue from lactating animals had virtually no incorporation of radioactivity into hsp-90 (lane 3, Fig. 3, and panel *d*, Fig. 4). The incorporation of [^{35}S]methionine into hsp-90 in the tissues from involuting mice (lane 4, Fig. 3, and panel *b*, Fig. 4) was similar to that seen with nulliparous mice.

Modulation of hsp-90 mRNA amounts during mammary development and differentiation

To define further the cellular basis for the modulation of hsp-90 amounts observed as a function of mammary development and differentiation, and to reconcile the observed discrepancy between a low relative rate of synthesis of hsp-90 by lactating tissues and an increased steady-state amount observed in immunoblot assays (Fig. 2*a*), the amounts of mRNA for hsp-90 were estimated in these tissues by using a homologous cDNA probe. As shown in Fig. 5(*a*), the maximal amount of mRNA was detected in tissues from pregnant mice and the lowest amount in tissues from lactating mice, which agreed with the relative rates of synthesis determined by [^{35}S]methionine-labelling experiments shown in Figs. 3 and 4. Fig. 5 also shows parallel analysis of the same RNA preparation for β-actin mRNA and whey-acidic-protein mRNA. As with hsp-90, β-actin mRNA was detected in tissues from all developmental states, and the overall profile appeared to resemble that seen with hsp-90 (Fig. 5*b*). However, quantification of these mRNA amounts by scanning densitometry revealed that the ratio of hsp-90 mRNA to β-actin mRNA was relatively constant in tissues from all developmental states, except during pregnancy, when the concentration of hsp-90 mRNA was approx. 1.7 times that of β-actin (results not shown). In contrast with β-actin mRNA, the mRNA for whey acidic protein was barely detectable in tissues other than those obtained from pregnant and lactating mice, with the maximal amount being present in lactating tissue (Fig. 5*c*); it is well established that lactating mouse mammary tissues have the highest content of mRNA for whey acidic protein [28]. In separate experiments it was verified by Northern-blot analyses that the size of the RNA hybridizing with the labelled cDNA (H-19) probe corresponded to approx. 3.9 kb (Fig. 5*d*).

It is well documented that mammary development and differentiation occurring during pregnancy and lactation is accompanied not only by an increase in epithelial-cell proliferation but also by an increased accumulation of RNA by these cells. An analysis of the mammary tissues from various developmental states revealed that in tissues used in our studies there was an approx. 3-fold increase in total RNA per cell (unit DNA) during pregnancy and an 11-fold increase during lactation. Previous studies have documented that the increase in total RNA occur-

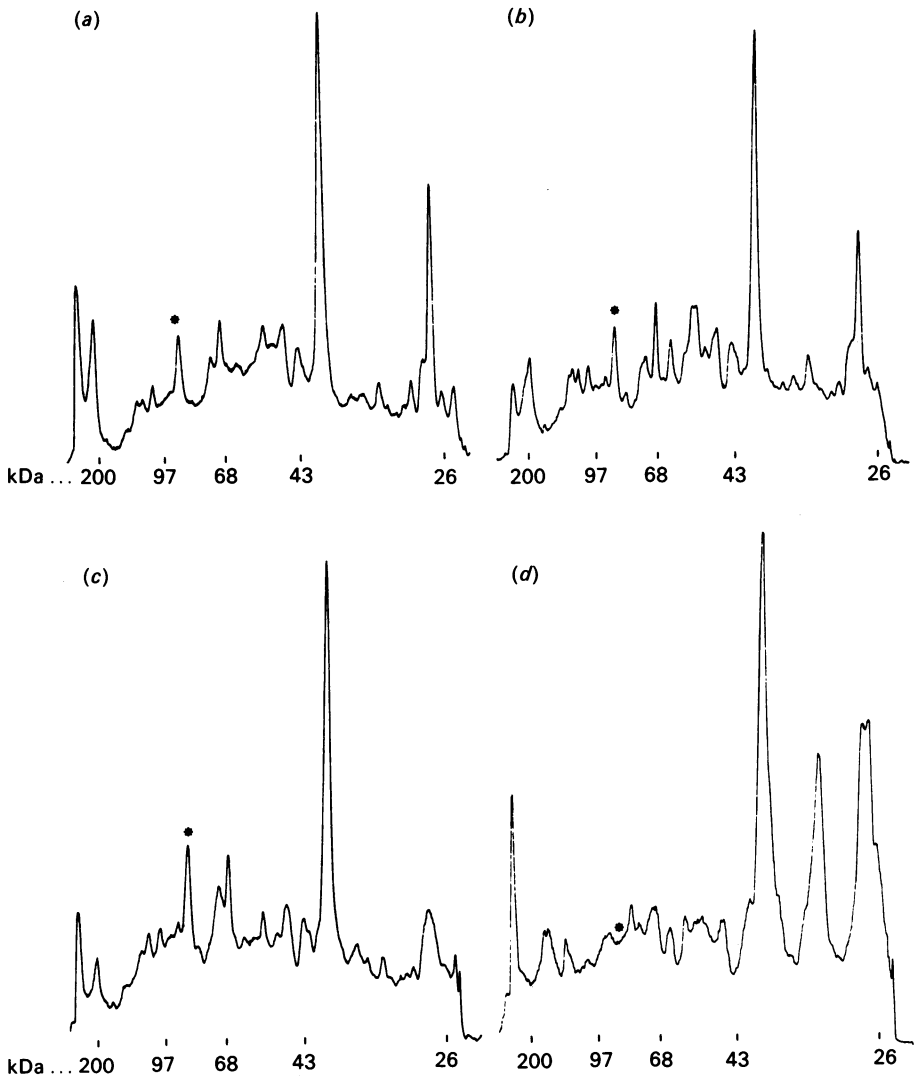

Fig. 4. Densitomer tracings of gels in Fig. 3

Lanes 1, 4, 2 and 3 of Fig. 3 correspond to panels (*a*)–(*d*) respectively in Fig. 4. The asterisk corresponds to the position of hsp-90.

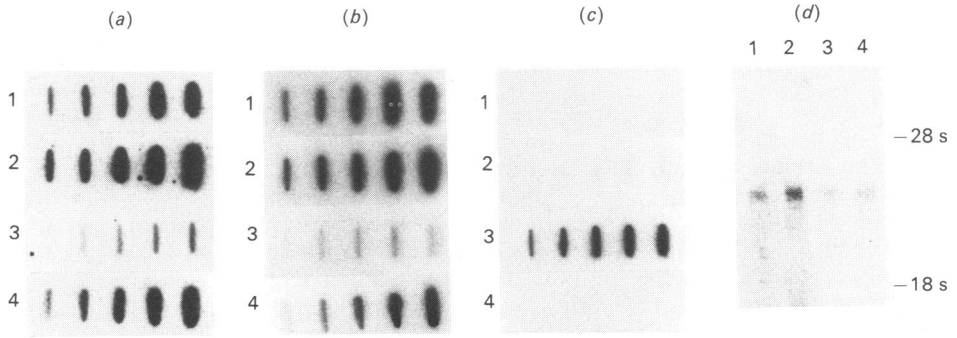

Fig. 5. Comparison of the amounts of mRNA for hsp-90, β-actin and whey acidic protein in mammary tissues from various developmental states

Total cellular RNA was prepared from various tissues and analysed for various mRNA contents by slot-blot and Northern-blot analyses as described in the text. In each panel, lanes 1, 2, 3 and 4 respectively correspond to RNA isolated from tissues of nulliparous, pregnant, lactating and involuting animals. Panels (*a*), (*b*) and (*c*) represent the slot-blot analyses for hsp-90, β-actin and whey-acid-protein mRNA respectively; in each blot 2.5, 5, 7.5, 10 and 15 μg of RNA (left to right) was loaded per slot. Panel (*d*) represents the Northern-blot analyses for hsp-90 mRNA.

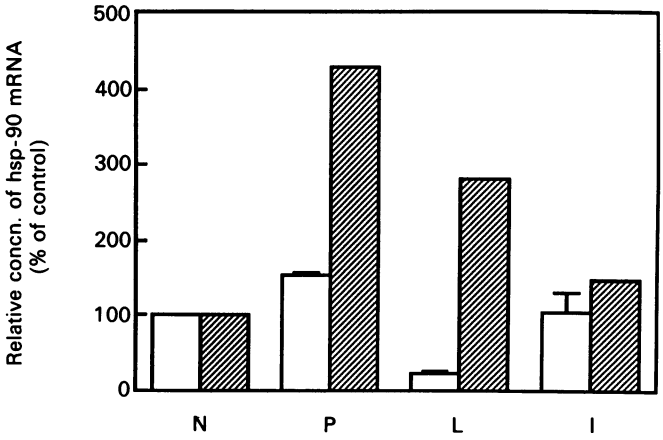

Fig. 6. Quantification of the hsp-90 mRNA in mammary tissues from various developmental states

Mammary tissues from various developmental stages were analysed for hsp-90 mRNA exactly as described for Fig. 5. For quantification, the autoradiographs were scanned and the data are expressed in terms of relative intensity. The 100% control value represents the scanning intensity observed with RNA from mammary tissues of nulliparous mice: □, relative scanning intensity among various tissues normalized to total cellular RNA; ▨, that normalized to total cellular DNA. The data represent means ± S.E.M. of three separate experiments. Abbreviations: N, nulliparous, P, pregnant; L, lactating; I, involuting.

ring during pregnancy and lactation is also accompanied by a selective increase in mRNA content for milk proteins such as casein and whey acidic protein [28,29], as also shown in Fig. 5(*c*). This raised the possibility that the lowest amount of hsp-90 mRNA found in lactating mammary tissue (Fig. 5) might have resulted from a dilution of this mRNA in the assay by the milk-protein mRNAs, which constitute the majority of mRNA during this developmental stage, and might not necessarily be due to a lesser amount present per cell. To verify this possibility, the data for hsp-90 obtained from experiments similar to those shown in Fig. 5 were analysed quantitatively in terms of both total RNA and DNA. These analyses, shown in Fig. 6, reveal that, although there are no qualitative differences in the profiles of hsp-90 mRNA, there is a major quantitative difference depending on whether the data are normalized to total RNA or DNA.

DISCUSSION

In the present studies we have examined the modulation of hsp-90 in murine mammary tissues and find that the amounts of both protein and mRNA are regulated as a function of development and differentiation. Overall the amount of mRNA corresponds to the amount of protein in tissues from each developmental state, but there are some significant differences between tissues isolated from pregnant and lactating animals. On the basis of either total RNA or DNA, it is during pregnancy that the tissues have the highest content of hsp-90 mRNA and the highest rate of [^{35}S]methionine incorporation into the protein. An analysis for the poly(A) content in three to four separate preparations of total RNA revealed essentially no differences between the tissues from nulliparous and pregnant mice, whereas during lactation there was an approx. 20% increase; the poly(A) content [estimated by hybridization to poly(U)] of RNA isolated from tissues of nulliparous mice was $1.66 \pm 0.08\,\mu$g/ml (mean ± S.E.M.), $1.77 \pm 0.09\,\mu$g/mg for tissues from pregnant mice and $2.21 \pm 0.03\,\mu$g/mg from lactating mice. These findings are in agreement with previous observations that the ratio of polyadenylated RNA to total RNA in mammary glands remains essentially unchanged until late pregnancy [29]. Therefore from our studies it would appear that during pregnancy there is a selective accumulation of hsp-90 mRNA. In contrast with tissue from pregnant animals, the highest amount of protein is present in lactating mammary tissue, which also has the lowest rate of synthesis. A lower rate of synthesis of hsp-90 during lactation is perhaps not unique to this protein, since in this developmental state mammary tissue is almost exclusively engaged in the synthesis of milk proteins. In this context, it should be noted that the [^{35}S]methionine-labelling experiments cannot yield precise quantitative information about the relative rates of protein synthesis, owing to certain inherent problems associated with this assay, e.g. dilution of the labelled compound to varying degrees by different tissues and hence possible differences in the specific radioactivity of the label. In any case our data indicate that, as compared with tissues from other developmental states, the tissue concentration of hsp-90 may be increased during lactation, and this may help to explain some of our previous observations on mammary steroid-receptor activation and binding to DNA, as discussed below.

In previous studies we had observed that, whereas oestrogen receptors present in mammary-tissue extracts of nulliparous mice were readily able to undergo activation *in vitro* and bind to DNA, in lactating-mammary-tissue extracts this interaction of oestrogen receptors with DNA and chromatin was impeded [2,3]. In contrast with oestrogen receptors, glucocorticoid receptors present in the same two tissue extracts did not exhibit any difference with respect to their ability to undergo activation and bind to either DNA or chromatin [2,30]. A quantitative analysis of the amounts of hsp-90, oestrogen receptors and glucocorticoid receptors in mammary tissues from these two developmental states revealed the following. In mammary-tissue extracts from nulliparous mice, the oestrogen-receptor concentration is approx. 0.7 nM, whereas in tissue extracts of lactating mammary glands the receptors are present at approx. 1.0 nM. However, as shown in Fig. 5, tissue extracts of lactating mammary glands contain 5 times more hsp-90 than do those from nulliparous mice. Consequently, at least *in vitro*, the ratio of hsp-90 to oestrogen receptors is increased by approx. 5-fold in lactating-mammary-tissue extracts, and this in turn may facilitate a greater degree of association between oestrogen receptors and hsp-90 in these tissue extracts as compared with that occurring in tissue extracts of nulliparous mice. Since the association of hsp-90 with steroid receptors is known to retard the conversion *in vitro* of receptors into a form capable of binding to DNA [4–8], at least in part, this may explain the relative inability of oestrogen receptors in lactating-mammary-tissue extracts to bind to DNA as compared with that observed in tissue extracts of nulliparous mice. Since during established lactation there is an approx. 4–5-fold increase in mammary glucocorticoid

receptors as compared with that present in tissues from nulliparous mice [31], the ratio between hsp-90 and glucocorticoid receptors remains relatively constant during these two developmental states. This then may also account for the fact that, in lactating-mammary-tissue extracts, in contrast with oestrogen receptors, interaction of glucocorticoid receptors with DNA is not impeded.

At present we can only speculate on the mechanisms underlying the modulation of mammary hsp-90 gene expression. Since the principal hormones triggering mammary growth during pregnancy are oestrogen and progesterone, it is likely that, at least in part, the increased accumulation of hsp-90 mRNA found in mammary tissues during pregnancy is due to an increased rate of synthesis of hsp-90 associated with steroid-dependent growth of the tissue. The higher content of hsp-90 mRNA and protein found in lactating mammary glands as compared with control tissues from nulliparous mice, however, cannot be simply due to an oestrogen-dependent phenomenon, since the tissues in this developmental state are insensitive to oestradiol [1]. It is known that, in addition to hormones, the developmental regulation of mammary gene expression can also be influenced by the extracellular-matrix components, resulting in an increased steady-state accumulation of milk proteins and their corresponding mRNAs [32–37]. This effect of the matrix components has also been shown to be far greater on protein turnover than on the steady-state accumulation of corresponding mRNAs [36]. Finally, it is to be noted that the amount of endogenous proteases such as plasminogen activator is also much lower in lactating mammary glands than in tissues from non-lactating animals [38], and trypsin inhibitors have been shown to be present in milk [39]. Thus the increased stability of hsp-90 in lactating mammary glands may be the result of several physiological processes associated with lactation. A clearer elucidation of the cellular mechanisms underlying mammary hsp-90 regulation must therefore await further studies.

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REFERENCES

1. Shyamala, G. (1984) Prog. Cancer Res. Ther. **31**, 293–308
2. Gaubert, C.-M., Carriero, R. & Shyamala, G. (1986) Endocrinology (Baltimore) **118**, 1504–1512
3. Shyamala, G., Singh, R. K., Ruh, M. F. & Ruh, T. S. (1986) Endocrinology **119**, 819–826
4. Joab, I., Radanyi, C., Renoir, J. M., Bouchou, T., Catelli, M. G., Binart, N., Mester, J. & Baulieu, E. E. (1984) Nature (London) **308**, 850–853
5. Sanchez, E. R., Toft, D. O., Schlesinger, M. J. & Pratt, W. B. (1985) J. Biol. Chem. **260**, 12398–12401
6. Mendel, D. B., Bodwell, J. E., Gametchu, B., Harrison, R. W. & Munck, A. (1986) J. Biol. Chem. **261**, 3758–3763
7. Baulieu, E. E. (1987) J. Cell Biochem. **35**, 161–174
8. Catelli, M. G., Binart, N., Jung-Testas, I., Renoir, J. M., Baulieu, E. E., Feramisco, J. R. & Welch, W. J. (1985) EMBO J. **4**, 3131–3135
9. Topper, Y. J. & Freeman, C. S. (1980) Physiol. Rev. **60**, 1049–1106
10. Baez, M., Sargan, D. R., Elbrecht, A., Kulomaa, M. S., Schulz-Zarucki, T., Tsai, M.-J. & O'Malley, B. W. (1987) J. Biol. Chem. **262**, 6582–6588
11. Ramachandran, C., Catelli, M. G., Schneider, W. & Shyamala, G. (1988) Endocrinology (Baltimore) **123**, 956–961
12. Morange, M., Diu, A., Bensaude, O. & Babinet, C. (1984) Mol. Cell. Biol. **4**, 730–735
13. Barnier, J. V., Bensaude, O., Morange, M. & Babinet, C. (1987) Exp. Cell Res. **170**, 186–194
14. Catelli, M. G., Binart, N., Feramisco, J. R. & Helfman, D. (1985) Nucleic Acids Res. **13**, 6035–6047
15. Catelli, M. G., Radanyi, C., Renoir, J.-M., Binart, N. & Baulieu, E. E. (1988) J. Cell. Biochem. Suppl. **12D**, 276
16. Riehl, R. M., Sullivan, W. P., Vroman, B. T., Bauer, V. J., Pearson, G. R. & Toft, D. O. (1985) Biochemistry **24**, 6586–6591
17. Ziemiecki, A., Catelli, M. G., Joab, I. & Moncharmant, B. (1986) Biochem. Biophys. Res. Commun. **138**, 1298–1307
18. Legagneux, V., Quélard, C., Morange, M. & Bensaude, O. (1988) J. Cell. Biochem. Suppl. **12D**, 280
19. Laemmli, U. K. (1970) Nature (London) **227**, 680–685
20. Towbin, H., Staehelin, T. & Gordon, J. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 4350–4354
21. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) Molecular Cloning, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
22. Meinkoth, J. & Wahl, G. (1984) Anal. Biochem. **138**, 267–284
23. Ceriotti, G. A. (1952) J. Biol. Chem. **198**, 297–303
24. Schneider, W. C. (1957) Methods Enzymol. **3**, 680–684
25. Bradford, M. (1976) Anal. Biochem. **72**, 248–254
26. Legagneux, V., Dubois, M.-F., Morange, M. & Bensaude, O. (1988) FEBS Lett. **231**, 417–420
27. Lindquist, S. (1986) Annu. Rev. Biochem. **55**, 1151–1191
28. Hennighausen, L. G. & Sippel, A. E. (1982) Eur. J. Biochem. **125**, 131–141
29. Rosen, J. M., Woo, S. L. C. & Comstock, J. P. (1975) Biochemistry **14**, 2895–2903
30. Ruh, M. F., Singh, R. K., Ruh, T. S. & Shyamala, G. (1987) J. Steroid Biochem. **28**, 581–586
31. Shyamala, G. (1982) in Hormonal Regulation of Experimental Mammary Tumors (Leung, B. S., ed.), vol. 1, pp. 245–286, Eden Press, Montreal
32. Emerman, J. T. & Pitelka, D. R. (1977) In Vitro **13**, 316–328
33. Wicha, M. S., Lowrie, G., Kohn, E., Bagarandess, P. & Mahn, T. (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 3213–3217
34. Suard, Y. M. L., Haluptte, M. T., Farinon, E. & Kraehenbuhl, J.-P. (1983) J. Cell Biol. **96**, 1435–1442
35. Li, M. L., Aggeler, J., Farson, D. A., Hatier, C., Hassell, J. & Bissell, M. J. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 136–140
36. Blum, J. L., Zeigler, M. E. & Wicha, M. S. (1987) Exp. Cell Res. **173**, 322–340
37. Chen, L. H. & Bissell, M. J. (1987) J. Biol. Chem. **262**, 17247–17250
38. Ossowski, L., Biegel, D. & Reich, E. (1979) Cell **16**, 929–940
39. Weström, B. R. & Carlsson, L. C. T. (1976) Int. J. Biochem. **7**, 41–47

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